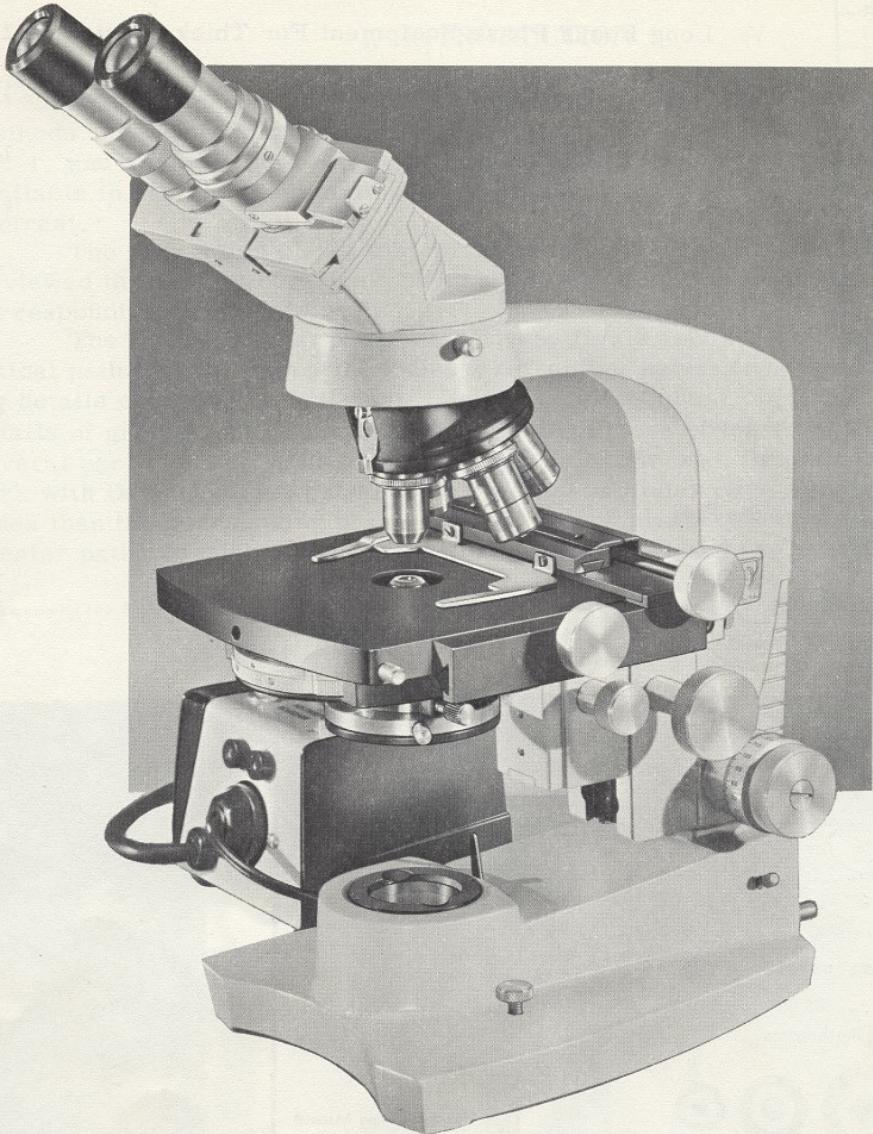


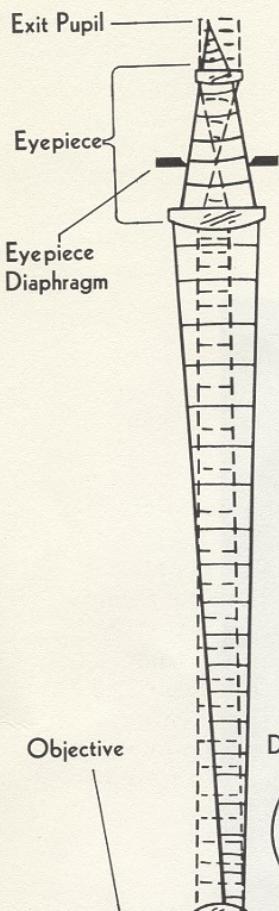
PHASE MICROSCOPE EQUIPMENT
REFERENCE MANUAL



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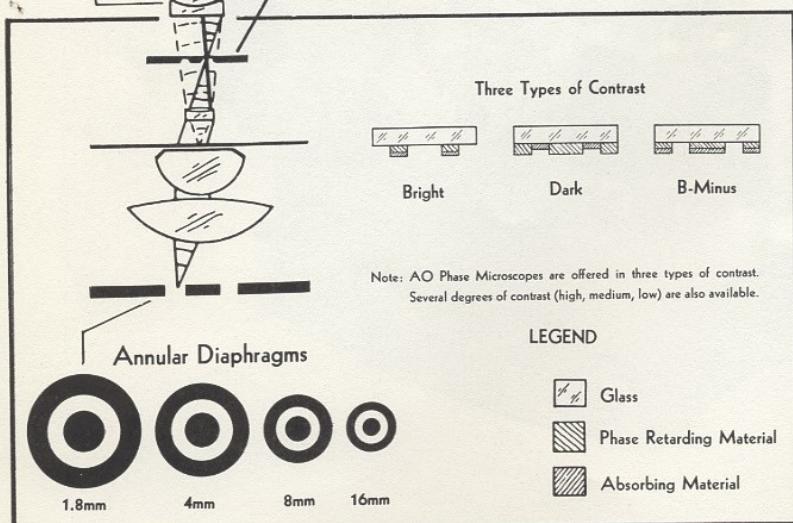
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Fig. 1.



DIRECTIONS

★ AO-Spencer Phase Microscopy Equipment ★

by Oscar W. Richards, Ph.D

CHIEF BIOLOGIST

These instructions are provided to help you use the phase microscope. It actually is quicker and easier to adjust the equipment correctly than to try for an improper compromise. Rereading the instructions, as necessary to keep them in mind will conserve time and increase accomplishment.

I. INTRODUCTION

Phase microscopy reveals details in specimens too transparent for other methods. A Phase Microscope requires an annular diaphragm for the condenser, fig. 1, and an objective containing a diffraction plate. The accessories are available in various combinations, for convenience of use and flexibility of contrast.

The diffraction plate¹ may be seen as a grayish ring when the objective is viewed through its back lens. The area of this ring is illuminated by the corresponding annulus.¹

The Dark Contrast phase objectives, fig. 2, show details of greater optical path (optical path = thickness x refractive index) darker than surrounding details of lesser optical path. Bright Contrast phase objectives show the details of greater optical path as brighter than those of lesser path. When several particles, as in an emulsion, appear bright with Bright Contrast and dark with Dark Contrast phase objectives the particles have a higher refractive index than the surrounding phase. The B-minus phase objectives also show the greater paths as darker than lesser path differences and are preferred for examining

¹ Sometimes the diffraction plate and annulus are colloquially called phase plate and annular respectively.

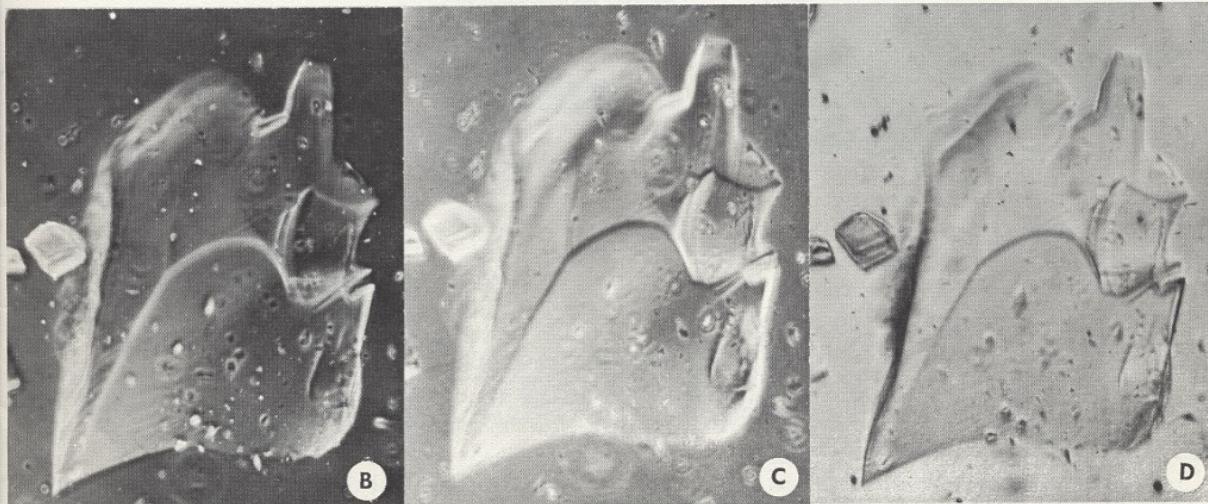


Fig. 2 — Glass Fragment mounted in balsam, 300X. (Refractive index, glass 1.52, balsam 1.54). A. Brightfield.
B. Bright Contrast Phase, C. Dark Contrast Phase, and D. B-minus Dark Contrast Phase Photomicrograph.

inclusions within other material and for slightly absorbing specimens (light pigmentation or faded colors). Three contrast levels, low, medium, and high, are available for bright and dark contrast; and low and medium contrast for B-minus phase objectives.

Explanation of the theory, instructions, and procedures for the use of the Phase Microscope for: refractive index determinations, contrast variation, image reversal from control of the mounting medium, combining color and phase contrast, dispersion optical staining, replicas, use with other methods of microscopy, stereophotomicrography; and biological, medical and industrial applications will be found in the references cited in Section VIII.

II. ILLUMINANT

NOTE: Remove any ground or diffusing glass from the lamp before focusing the lamp filaments or the field stop. A ground glass between the lamp and the microscope is helpful when centering the phase condenser. A ground glass should be used only with the lower magnification objectives, but not for critical microscopy with the 4mm and 1.8mm phase objectives. When the light is too bright it can be controlled with neutral density filters placed between the lamp and the microscope.

For best results a research type lamp with a condensing lens and iris should be used, such as the AO No. 735. Built-in illumination as the AO No. 600, is suitable for visual use and many photographic applications. Either Köhler or critical illumination may be used for phase microscopy; the Köhler method is better with filament lamps.

A. The No. 735 Illuminator.

Focus the filament image (partial closing of the iris diaphragm helps) on a wall about one or two feet from the lamp and adjust the 3 centering screws on the back of the lamp housing, fig. 3, if necessary, to place the image from the mirror of the filaments between and in the same plane as the filament image.

B. The No. 370 Illuminator.

Centering is done by loosening the screw that holds the socket in the housing, fig. 4, and tilting, turning, and raising or lowering the lamp as required to center the lamp to the optical axis of the condensing lenses and to intermesh the reflected image of the filaments with the image of the filaments. The housing must be level and at right angles to the screen, neither tilted up or down, when centering is done.

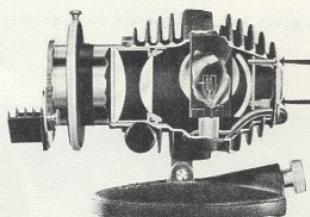


Fig. 3 - AO No. 735 Lamp showing screws for adjusting the mirror.

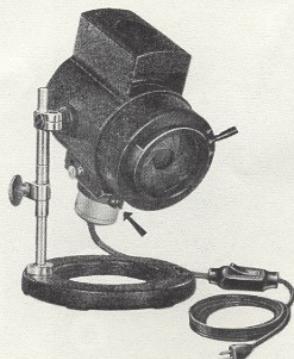


Fig. 4 - AO No. 370 Lamp showing set screw and clamp for adjusting the lamp position by moving the lamp socket.

C. With built-in illumination or other sources, adjust the lamp so that the field is seen to be evenly illuminated, the iris diaphragm of the illuminator in focus with the specimen and the full back aperture of the objective (as seen with the Phase Telescope or Bertrand lens) is filled as uniformly as possible with light.

III. BASIC INSTRUCTIONS

The essential adjustments of the phase microscope require only that (1) the phase condenser is centered to the objective, that (2) the image of the annulus matches (superimposes on) the diffraction plate exactly with no light leakage and that (3) the illumination be uniform and sufficient and condenser properly focused.

A. The Telescope or Bertrand lens

The diffraction plate within the phase objective is too small to be seen easily. For convenient adjustment it is examined with the aid of the telescope, fig. 5, which is inserted in place of the ocular, or with a Bertrand lens, fig. 6, that slides in and out of the body tube. The Bertrand lens with the ocular forms a telescope and can be raised or lowered to focus on the diffraction plate. The telescope is focused by sliding the upper part in or out as required. This built-in telescope (Bertrand lens) permits a rapid check of the adjustment at any time and is especially useful when using long focus equipment. Either can also be used to advantage with the brightfield microscope for centering the condenser and illumination.



Fig. 5 — The Phase Telescope



Fig. 6 — The Bertrand Lens.

B. Adjustment

1. Place the illuminator (properly adjusted, Section II) about 6 inches in front of the microscope, direct the light to the center of the mirror (flat side) and focus the lamp filament onto the iris diaphragm of the microscope condenser. Focus the microscope on a specimen and then focus the microscope condenser until the lamp iris (closed enough to be seen, or the mirror slightly tilted until the edge of the lamp iris is seen) is in focus with the specimen. Open the lamp iris.

2. The equipment must be centered or its centration checked. Therefore, remove the ocular and insert the telescope, or slide the Bertrand lens into the optic axis of the microscope (Sec. III A.) and focus it on the diffraction plate in the objective. The annulus must be removed by turning the Phase Turret condenser to the "O" (open) position, fig. 9A or by actually taking the annulus out of the single condensers. Close the microscope condenser iris until it surrounds a part of the diffraction plate and adjust the centering screws of the condenser so that the iris appears concentric with the diffraction plate, fig. 7 B. Rotate the turret to bring the proper annulus into view, or insert it in the single unit condenser, and adjust the centering screws for the annulus until the annulus appears concentric with and superimposes on, or matches the diffraction plate, fig. 8 C. Failure to match indicates the attempted use of the wrong annulus, failure to focus on the specimen, or failure to focus the condenser. (See the following sections for detailed centering adjustments.) Slide the Bertrand lens out, or replace the telescope with an ocular and the Phase Microscope is ready for use as soon as the light is centered by slightly adjusting the microscope mirror.

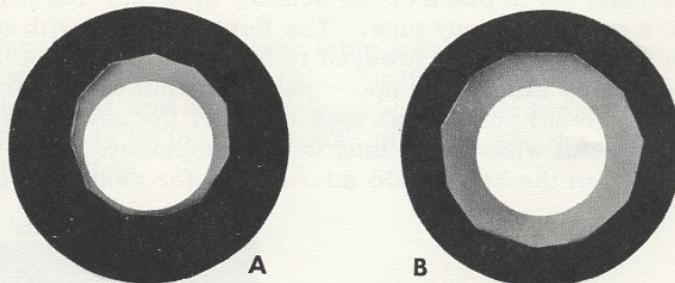


Fig. 7 — Appearance of the objective aperture when the microscope condenser is: A. out of center, and B. properly centered.

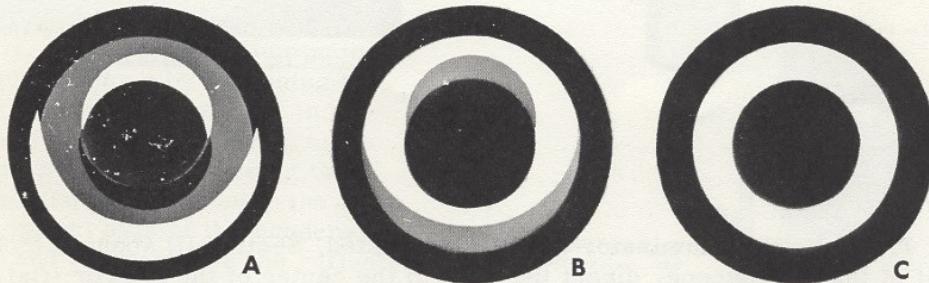


Fig. 8 — Appearance of the objective aperture when the annulus is out of center with A. dark contrast and B. bright contrast diffraction plates; and C. properly matched to the diffraction plate. (See text)

C. B-minus Phase Objectives

The low contrast B-minus objective has no absorbing materials on the diffraction plate and is not seen with the telescope. First center the annulus with a bright or dark phase objective of the same magnification. Then place the B-minus objective in place and slightly turn all the centering screws until best contrast is obtained.

D. Illumination

The iris diaphragm of the illuminator is often called a field stop as it is seen in the field (in focus with the specimen) and when properly adjusted it limits the illumination to the field of view. Opening it further merely decreases visibility by adding glare from light scattered within the microscope.

A bluish colored central area seen in the field of view indicates that the microscope condenser is focused too high. Likewise a brownish area around the edge of the field indicates that the microscope condenser is focused too low. Uneven color at the edges indicates either that the lamp is out of center in the illuminator, the lamp beam is not on the center of the mirror, or that the condenser is not centered.

No color filter is required with AO-Spencer Phase Equipment. A green filter or other color may be used if desired. For visual use, an Interference Filter with transmission at 555mu, will give better results than broad band filters.

E. General

Focusing a transparent specimen is often difficult before the phase system is properly aligned. Visibility can be aided by partially closing the microscope iris or by the use of an annulus for a higher magnification objective (iris fully open) which gives a dark-field effect.

Irregular, lens shaped (hollow ground slides) or wedge shaped preparations upset the alignment of the phase system and should not be used. With only a small wedge, as when the cover glass is not quite parallel with the slide, it may be necessary to recenter the condenser each time the specimen is moved in order to maintain good phase contrast. Flat bottom Hemacytometers (AO No. 1475) are available for phase microscopy.

Fresh or liquid preparations should be sealed to prevent evaporation. Preparation methods are described in the references in Section VIII.

Long focus equipment will require more adjustment unless the specimen mounts are flat, parallel and of good optical quality.

Any light leakage around the diffraction plate degrades the image. The adjustments are easy and certain as the circles of the diffraction plate and of the image of the annulus are made to fit each other. With standard preparations and proper care a given microscope once centered will remain in adjustment for some time.

IV. SPENCER TURRET PHASE CONDENSER, figs. 9, 10

The Turret Condenser contains four centerable annuli and one open space, fig. 10 E, any one of which can be positioned within the condenser by turning the knurled ring. Four objectives and their annuli may be properly aligned, and readily changed, which is a convenience when more than one magnification or contrast is required.

The long focus equipment described in Section V ABC and E may be used with the Turret Condenser for use with specimen mounts thicker than standard microscope slides. (See also Section III D)

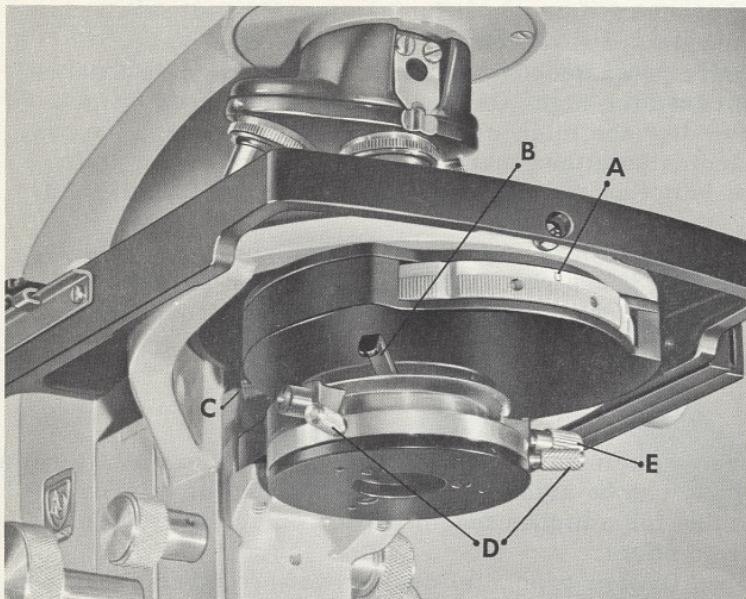


Fig. 9 — The AO Spencer Turret Condenser catalog number 1000250 showing:
A. turret; B. lever for iris diaphragm; C. opening (port) for centering wrench;
D. centering screws for centering condenser; E. retaining screw.

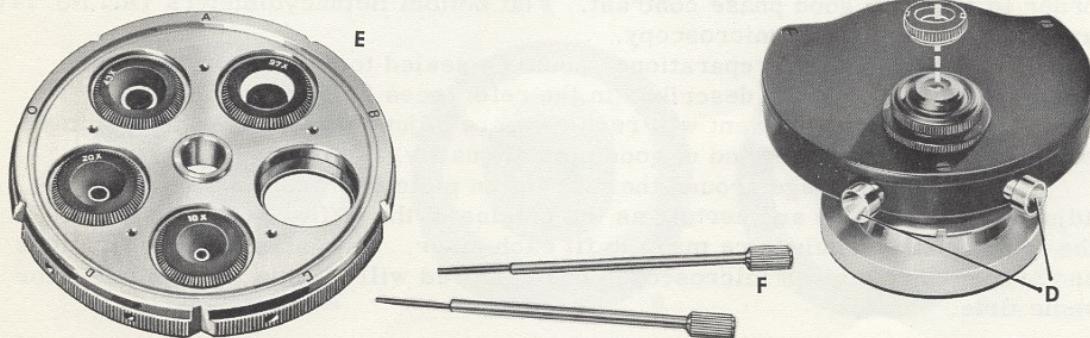


Fig. 10 — AO Spencer Turret Condenser showing: AA', condensing lenses; B.
wrench; C. annular diaphragm; D. ports or openings, for centering wrenches;
E. turret plate removed from housing to show annular diaphragms; F. centering
wrenches.

A. Directions for Use

1. Remove the condenser from the fork mount.

2. Remove the left hand screw (microscope arm toward you) at the spring detent of the fork and replace it with the knurled stainless retaining screw, fig. 9 E, furnished with the unit.

3. Insert the Phase Turret Condenser making sure that the slot fits into the back of the fork and that the knurled screw is tightened firmly. When this is done carefully the condenser may be removed and replaced to the same central position.

If the turret does not center, rotate it until it fits into the locking slot, before tightening the screw.

4. Direct the light from the lamp (properly adjusted - Section II) to the center of the microscope mirror and focus the lamp filaments (ground glass, if any, removed) onto the iris diaphragm of the Turret.

5. Focus on a specimen, close the lamp iris and focus the microscope condenser so that the lamp iris is in focus with the specimen. Adjust the mirror until the image of the iris is centered and open the lamp iris.

When the condenser cannot be raised enough to focus the lamp iris with the specimen the limit stop should be adjusted. This is done on the Phasestar by inserting the 1/16" Allen wrench up through the opening in the bar under the substage rack, into the adjusting screw and turning it until the substage can be raised sufficiently. With the older models the lock nut on the stop pin at the top of the substage is loosened and the pin screwed in until the condenser movement is adequate.

6. Remove the ocular and insert the telescope, fig. 5, in place of the ocular; or slide the Bertrand Lens, fig. 6, to the in position and focus the telescope by moving its upper part, or raising or lowering the mount of the Bertrand Lens until the diffraction plate in the phase objective is seen clearly.

7. Turn the knurled disc of the turret to the position "O", fig. 9 A. Close the Turret Condenser iris until it is near the ring on the diffraction plate, fig. 7 AB, and center the iris with the centering screws fig. 9 D, as shown in fig. 7 B.

8. Turn the disc until the diffraction plate for the objective in use is in place and center it with the special centering wrenches, fig. 10 F, by inserting the wrenches into the ports, fig. 10 D. By turning these slightly as they are inserted you will feel them enter into the adjusting screws of the annulus mount. Center the annulus to match the diffraction plate as in fig. 8 ABC. Remove the wrenches.

Note: Usually the 16mm annulus is in the A position, the 8mm in the B, the 4mm in the C, and the annulus for the 1.8mm phase objective is placed in the D position. When other combinations of objectives are on the nosepiece, the arrangement may be different.

9. Remove the Bertrand Lens, or replace the telescope, with standard ocular. Recenter the mirror by tilting and set the lamp iris until the field is just filled with light. (See Section III D). The microscope is now ready for use with that objective.

10. Center the other phase objectives and annuli by repeating steps 5 to 10.

11. Note: With B-minus objectives follow directions in Section III C.

For ordinary phase microscopy, it is not necessary to use immersion oil between the slide and the condenser. For critical observation immersing the condenser will give a little more light and slightly better imagery with the 1.8mm objective.

12. Once the annuli have been centered to a given set of objectives they will remain centered for considerable periods of time with careful use of the microscope. Centration should be checked from time to time for critical observation, or photomicrography. (See Section III B2)

When different phase objectives are placed on the nosepiece, recentering of the annuli (paragraphs 5 to 10) will usually be required and if the new objective is of different magnification than the one removed, the annulus must also be changed. Directions for changing the annuli are given in Section V E1, 2.

When the specimen mount is not flat and parallel the Turret may need recentering each time the specimen is moved to maintain better contrast.

V. LONG FOCUS PHASE EQUIPMENT FOR THICK SPECIMENS AND/OR CHAMBERS

The standard turret and single condensers are made for standard thickness microscope slides. For thicker preparations special equipment is necessary. Note, however, that hollow ground, curved or wedged mounts are rarely suitable for phase microscopy. Special Hemacytometers, AO No. 1475, without the concave base are available for the Phase Microscope.

Best results with long focus equipment are obtained when the specimen preparation has parallel walls of good grade glass with flat surfaces. When this is not attained, moving the specimen will upset the adjustment (due to the wedge or prism effect of the mount) and the centering procedures must be repeated for good seeing each time the specimen is moved. With specimens growing in test tubes, etc. only the cells at the very top inner surface of the mount will be seen clearly.

With properly prepared specimens (thinner the better) good contrast can be obtained at all magnifications of the phase microscope. With less suitable specimens it may still be possible to get usable contrast by slightly refocusing the condenser to obtain as good a match between the annulus and the diffraction plate as possible as seen with the telescope. Some experimenting can lead to a compromise that is helpful and better than no phase contrast, but best contrast is attained only when the conditions of Section III are fully utilized.

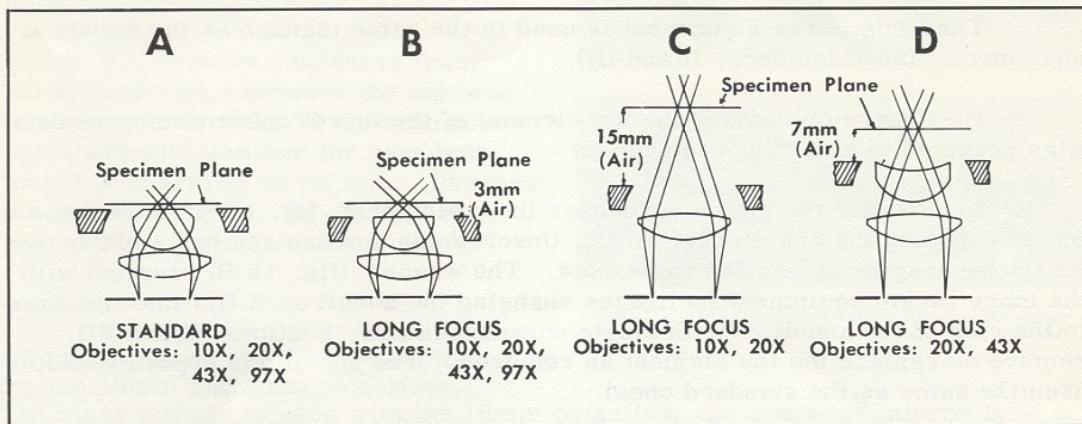


Fig. 11 — Diagrams of phase condensers. A. For standard thickness slides. B. With reduced thickness top lens. C. With top lens removed. D. With N.A. 0.66 top lens. B, C. and D. are long working distance systems for thicker specimens.

A. With preparations of somewhat greater thickness (but not exceeding 4-1/2mm of glass or equivalent optical path²) a reduced thickness top element #1000303 is used instead of the regular top lens of the phase condenser and the standard annuli are used in the condenser, fig. 11 B.

B. Increased working distance equal to 15mm in air² can be obtained with a 16mm 10X and 8mm 20X phase objectives by removing the top element, fig. 10A, of the condenser, fig. 11 C, and using special annuli as follows:

For the 16mm 10X use the #1000110 annulus with the green rim.

For the 8mm 10X use the #1000106 blue rim annulus with the larger central stop (12mm diameter).

² One mm of air equals 1.33mm of water or 1.52mm of crown glass. These ratios (refractive indices) can be used to obtain the equivalent working distance when the specimen includes more than one medium.

C. An increased working distance equal to 7mm air path² may be obtained for the 8mm 20X and the 4mm 43X phase objectives by replacing the top element of the regular phase condenser with the #1000306 N.A. 0.66 top element, fig. 11 D, and using special annuli as follows:

With the 8mm 20X use the #1000113 blue rim annulus with the smaller central top (6mm diameter).

With the 4mm 43X use the #1000107 annulus with the yellow band.

D. Or use the Special Long Focus Equipment #1000310. (Section V F).

E. Instruction for Long Focus Equipment used with the Standard Phase Condensers.

The Long Focus Equipment is used in the same manner as the standard equipment. (See also Secs. II and III).

1. Change or remove the top element of the turret condenser or replace it as required in sections A to C above.

2. Remove the entire condenser (both elements, fig. 10 AA') from the turret exposing the annuli, fig. 10 C. Unscrew the annulus and in its place insert the long focus annulus to be used. The wrench (fig. 10 B) supplied with the more recent equipment facilitates changing the annuli as it fits into the slots in the ring of the annuli. (With single unit condensers, Sections VI and VII, remove or replace the top element as required. The annuli are separate and used the same as the standard ones).

3. Replace the lenses and put the condenser on the stand making sure it slides back into the locking slot and that the retaining screw is tight.

4. Direct the light from the illuminant to the center of the mirror and into the microscope.

5. Focus on the specimen and focus the microscope condenser until the field stop (lamp iris) on the illuminant is in focus with the specimen and the field is evenly lighted.

6. Center the condenser until its iris is concentric with the diffraction plate in the objective as seen with the telescope or Bertrand Lens (fig. 7 AB).

7. Turn the turret until the proper annulus is in place and center it to the diffraction plate as in Section IV A8.

8. Should the annulus and the diffraction plate not match exactly refocus the microscope condenser slightly until the best match is obtained, but not enough to make the illumination of the field uneven. (Section II D).

² One mm of air equals 1.33mm of water or 1.52mm of crown glass. These ratios (refractive indices) can be used to obtain the equivalent working distance when the specimen includes more than one medium.

F. Long Working Distance Condenser #1000310, fig. 12.

The working distances provided by this single unit condenser are 15mm air path² (or its equivalent of 22mm glass) for the 16mm 10X and the 8mm 20X phase objectives, 11mm for the 4mm 43X and 7mm for the 1.8mm 97X phase objectives. The annuli for the unit are mounted in rings with convenient handles, fig. 12 B, for inserting them into a slot in the lower front side of the condenser.

1. Remove condenser from the microscope. Remove the top lens of the condenser, fig. 12 C, by unscrewing it and note whether the next lens mount is engraved on its rim. Unscrew the second element, fig. 12 D, if no engraving is seen and turn it over.

A. Replace the second element only with the engraving down as indicated for the 16mm and 8mm objectives.

The plane mirror is used with the 16mm objective, the concave³ mirror is used with the 8mm phase objective.

B. For use with the 4mm 43X or 1.8mm 97X objective, be sure that the second element of the condenser, fig. 12D, is turned engraving up as indicated by the engraving on it. Replace the top element, fig. 12 C. Use the plane mirror.

2. Illuminate the microscope and focus the lamp iris in the plane of the specimen. With the telescope, or Bertrand lens center the iris diaphragm of this condenser to the diffraction plate of the phase objective, fig. 7 AB.

3. Insert the proper annulus and note whether or not it appears concentric to and fits the diffraction plate. If the fit is not good and much light leakage occurs, check to make sure the proper annulus (note engraving on the handle) is in use, or that the second lens element is properly orientated. A small lack of fit may be improved by racking the condenser slightly up or down, but not moving the condenser enough to give uneven lighting of the field. Should the annulus appear to be slightly off center in one direction, this may be due to a prismatic effect in the preparation and may be corrected by moving the annulus slightly off center in the condenser.

² One mm of air equals 1.33mm of water or 1.52mm of crown glass. These ratios (refractive indices) can be used to obtain the equivalent working distance when the specimen includes more than one medium.

³ This is one of the rare exceptions to the rule of using a plane mirror with a condenser.

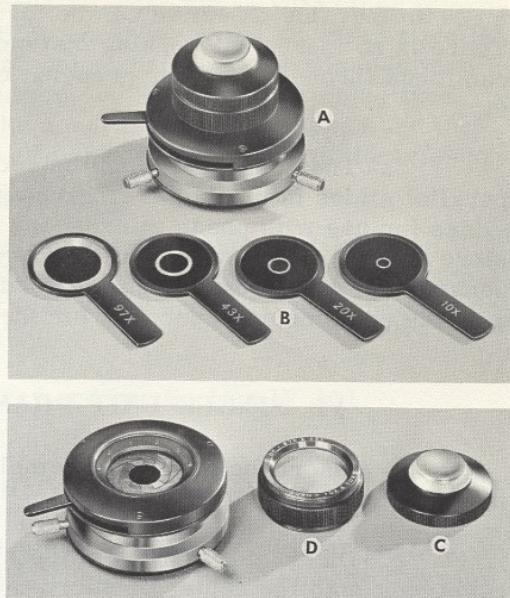


Fig. 12 — The 1000310 Long Working Distance AO Spencer Phase Condenser, A; and annuli, B; top element, C; lower element, D.

4. 1.8mm. Be sure to place immersion oil between the objective and the cover glass, but not on the top of the condenser. The preparation must be made so that this objective can be focused on it (No. 1 or 1-1/2 cover glass). There is no increased working distance of the objective, only the condenser can focus through a thicker preparation, such as a deep slide, Carrel flask, etc.

5. The condenser must be recentered each time a specimen with non-parallel slides is moved, i.e. hanging drop. Therefore, tissue flasks and slides for use with phase microscopy should be chosen of high quality material. More than usual practice is necessary for best results with the long focus condenser, but with proper handling good contrast and visibility can be obtained with the equipment, especially for the higher powers.

VI. SINGLE UNIT PHASE CONDENSER #1000135

1. Insert the annulus assembly into condenser.

2. Direct the light from the illuminant onto the center of microscope mirror and focus the filament image of the lamp at approximately the lower plane of condenser mount. (Section II)

3. Place a slide on the stage and focus on the preparation.

4. Focus the microscope condenser so that the lamp iris is in focus with the specimen and coincides to the edge of the field of view.

5. Remove the eyepiece and place the telescope in the ocular tube, or slide the Bertrand lens into the tube. Focus onto the diffraction plate in the objective and center the condenser annulus to the diffraction plate by means of the knurled centering screws. (figure 8)

6. Remove the telescope and replace the eyepiece. Recenter the mirror to obtain uniform illumination. The microscope is now ready for phase.

7. For B-minus objective, see Section IIIC.

8. The long focus accessories described in Section V A to E may be used with condenser #1000135.

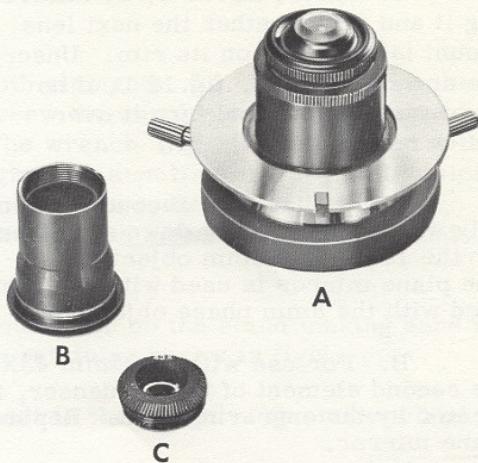


Fig. 13 - The 1000135 AO Spencer Single Unit Phase Condenser, A; removable holder for the annulus, B; and annulus C.

VII. PHOTOMICROGRAPHY

Pictures may be taken with the Phase Microscope as with other microscopes. Exposures with the B-minus will be about the same as with brightfield; dark and bright contrast phase will require somewhat longer exposures than brightfield depending on whether low, medium, or high contrast objectives are used. Be sure that the annulus and the condensers are centered and that there is no light leakage around the diffraction plate (as seen with the telescope, or Bertrand lens) for pictures of excellent detail and contrast.

Stereophotomicrographs are useful and may be made by tilting the specimen when the lower power objectives are used, or by the half aperture method for high powers. Bright contrast frequently shows better three dimensional detail than dark contrast.

NOTE: When oil immersion objectives are used see Section IV A11.

VIII. REFERENCES

*These references cover much of the literature to 1956. For later papers Biological Abstracts, (3815 Walnut St., Philadelphia 4, Pa.) and the Current List of Medical Literature (Government Printing Office, Washington, D. C.) may be consulted.

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